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Removing Selenite from Groundwater with an In Situ Biobarrier: Laboratory Studies

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Abstract. Laboratory biobarriers were evaluated for their ability to remove selenite from flowing groundwater. Microbial activity in aquifers is usually limited by substrate availability, and biobarriers stimulate microbial activity by providing a substrate; for these studies soybean oil was used. Water containing 10 mg L^{-1} selenite-Se was pumped through the biobarriers for 74 days and the amount present in the effluent monitored. The amounts remained high for the first 2 weeks of the study but then declined. From day 28 until the end of the study the amount of selenite-Se in the column effluents averaged 0.20 ± 0.04 mg L^{-1}, a decrease of approximately 98%. At the end of the study about half of the selenite-Se applied to the columns was recovered as immobilized selenium trapped by the biobarrier. This study suggests that biobarriers containing vegetable oil might be used as a process for removing selenite from contaminated groundwater.

Selenium, an essential trace element that in small amounts contributes to the normal growth and development of both humans and animals, is toxic in large amounts [32]. For this reason the US Environmental Protection Agency (USEPA) has set a limit of 0.05 mg L^{-1} for selenium in water consumed by humans [33]. Under aerobic conditions selenite is one of the principal forms of soluble selenium present in groundwater. In situ treatment may provide a low-cost approach for reducing the amount of selenite in groundwater. Normally, in deeper soils and in aquifers, microbial activity is restricted by substrate or electron donor availability [20, 29, 34]. In situ treatment functions by providing the needed electron donor. In situ treatment processes often involve the injection of a soluble carbon source, which can serve as a microbial electron donor, into the contaminated aquifer. The injected substrate stimulates microbial activity resulting in the degradation of contaminants that can serve as electron acceptors. Such applications, usually used for nitrate remediation, may also work for selenite remediation. However, when soluble substrates are used the expense of the storage tanks, pumps, and metering systems needed to deliver the substrate to the groundwater must be considered. In addition, with soluble substrates, aquifer plugging or biofouling has often been a major problem [8, 9]. Alternatively, insoluble electron donors can be used to form biobarriers. For shallow aquifers a biobarrier might be created by digging a trench across a contaminated aquifer and backfilling the trench with a permeable matrix containing the electron donor. A mixture of pea gravel and sand can be used for the permeable matrix and sulfur or an insoluble organic material can be used to provide the electron donor. The biobarrier would be positioned to intersect the contaminated aquifer before the water is extracted. Such barriers involve the cost associated with digging and backfilling of the trench but eliminate the need for storage tanks, pumps, and metering equipment and, because the substrate is dispersed over a large area, may also be less likely to plug or biofoul the aquifer [9].

In theory, as groundwater containing selenite flows through the biobarrier, microorganisms that are naturally present in the groundwater, or introduced microorganisms, would reduce the selenite to elemental selenium. Mechanisms by which this reduction might take place

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include: (i) the enzymatic reduction of selenite to selenium, which can take place under either aerobic or anaerobic conditions, and may be either a microbial detoxification process or a respiratory process [30] and (ii) abiotic reduction. At redox potentials of about 213 mV selenite reduction begins to occur by abiotic processes, though rapid reduction does not occur until −106 mV [22]. Also, the reduction of selenite to elemental selenium by sulfide formed by sulfate-reducing bacteria can occur [7, 8]. The interior of biobarriers contain large populations of denitrifying heterotrophic bacteria [14]. Under these conditions, it is likely that selenite would be reduced, by microbial activity, and perhaps by abiotic processes as well, to elemental selenium [31] and that selenite would be removed from the groundwater as the groundwater flows through the biobarrier. Elemental selenium is insoluble and the water leaving the biobarrier would contain less selenium than the water that entered the biobarrier. Once the selenite has been reduced to elemental selenium it should remain in this reduced state as long as the biobarrier continues to function because of the anaerobic conditions that exist within biobarriers [31, 35].

Sawdust, crop residues, sulfur, or vegetable oil coated onto the gravel and sand have been proposed as suitable microbial substrates for biobarriers designed for nitrate remediation [7, 16, 17, 23–25] and may also function in barriers designed for the removal of selenite from shallow aquifers. Deeper aquifers that are too deep for continuous trenching equipment would require a different approach. Here the injection of a vegetable oil emulsion might work best. Both laboratory [14, 18] and field [19] studies have shown that vegetable oil emulsions can be injected into soil to form stationary biobarriers. Past studies with vegetable oil have focused on groundwaters contaminated with nitrate, chlorate, perchlorate, or chlorinated solvents [11, 14, 18, 19]. The objective of the present investigation was to use biobarriers formed in laboratory columns to investigate the effectiveness of soybean oil as a substrate for the removal of a heavy metal, selenite, from flowing groundwater.

Materials and Methods

Sand columns. The columns used for this study were water-jacketed 150 mL glass columns 2.6 cm in diameter and 30 cm long. Columns were packed with 237 g of 30 grit (0.35 mm sieve size) quartz sand that had been coated with 2.5 g of soybean oil. Bulk density of the sand was 1.5 g cm^3 and pore volume was 68 mL. Columns were maintained at 15°C in the dark. Column effluents were collected three times per week and analyzed for pH, nitrate, nitrite, and selenite. In addition effluents were analyzed for COD on a weekly basis. Water supplied to the columns was a moderately hard reconstituted water, pH 6.5, containing $NaNO_3$, 121; $NaHCO_3$, 96; $CaSO_4 \cdot H_2O$, 60; $MgSO_4 \cdot 7H_2O$, 60; KH_2PO_4, 43.8; $NaSeO_3$, 22; $(NH_4)_2SO_4$, 5; KCl, 4.0; FeEDTA, 1.8; H_3BO_3, 0.5; $MnCl_2 \cdot 4H_2O$, 0.1; $ZnCl_2$, 0.1; $CuCl_2 \cdot H_2O$, 0.01; $MoCl_3$, 0.01 mg L^{-1}, respectively. This water had a hardness of 80–100 as mg L^{-1} $CaCO_3$ and an alkalinity of 60–70 mg L^{-1} as $CaCO_3$ [28]. The nitrogen content of the water was ammonia-N at 1 mg L^{-1}, nitrate-N at 20 mg L^{-1}, and the selenium content was 10 mg L^{-1} selenite-Se. The influent water was stored at 4°C and was replenished weekly. The influent water was in equilibrium with the laboratory air and no attempt was made to remove dissolved oxygen from the influent water supplied to the columns; measurements with an oxygen electrode indicated that the influent water normally contained 6–7 mg L^{-1} dissolved oxygen. Under these holding conditions there was no detectable oxidation of selenite to selenate. In order to assure the presence of a viable microbial population all columns were inoculated with a soil wash preparation [9] at the start of each study. The influent water was pumped upwardly through the columns at a flow rate of 1.8 mL h^{-1}.

Microcosms. Microcosms were 120 mL serum bottles containing 25 mL of the reconstituted water described above except that the medium was supplemented with 180 mg L^{-1} $NaNO_3$-N, 0.005% Tween-80, 1 g soybean oil, and $NaSeO_3$ as indicated. All microcosms received 0.25 mL of a soil wash preparation [9]. To make the system anaerobic the medium was purged with helium and a helium atmosphere was added to the microcosm bottles. Microcosms were capped with a rubber serum stopper and were incubated in the dark at 28°C while being shaken at 120 rpm on a rotary shaker. Samples were collected with a syringe after 7 days. Each treatment was replicated five times.

Analysis. For the analysis of total selenium in sand, 1 g samples from the sand columns were digested with nitric and perchloric acid and analyzed with inductively coupled plasma atomic absorption spectrometry as described in Spark [27]. The analysis was performed by the Colorado State University Soil, Water, and Plant Testing Laboratory.

For the analysis of dimethylselenide (DMSe) and dimethyldiselenide (DMDSe), gases that formed in the columns were caught in a trap installed in the stainless steel effluent lines running from each column. The trap was an inverted glass tube outfitted with a Teflon-lined rubber septum. Samples, 500 μL, of the effluent gas were collected with a glass and Teflon syringe at weekly intervals and analyzed for DMSe and DMDSe as described in Hunter and Kuykendall [15].

A high-pressure liquid chromatograph was used to estimate the nitrate and nitrite content of column effluents [14]. Ammonia was estimated by the phenate method [5]. A chromic acid digestion using kits procured from Chemetrics (Calverton, VA) was used to measure COD. pH was measured with a standard pH meter. Selenite was assayed colorimetrically by method 3500-Se D as described in Standard Methods for the Examination of Water and Wastewater [5]. Selenate was determined by ion chromatography.

Statistical comparisons. Statistical comparisons were made using the Instat computer program (GraphPad Software). Statistical comparisons presented in the text and figures are standard error of the mean computations.

Results

Nitrate and selenite removal by column biobarriers. Vegetable-oil-based biobarriers supplied with the simulated groundwater containing both nitrate and selenite were found to remove both oxyanions from flowing water. Nitrate removal occurred rapidly. The

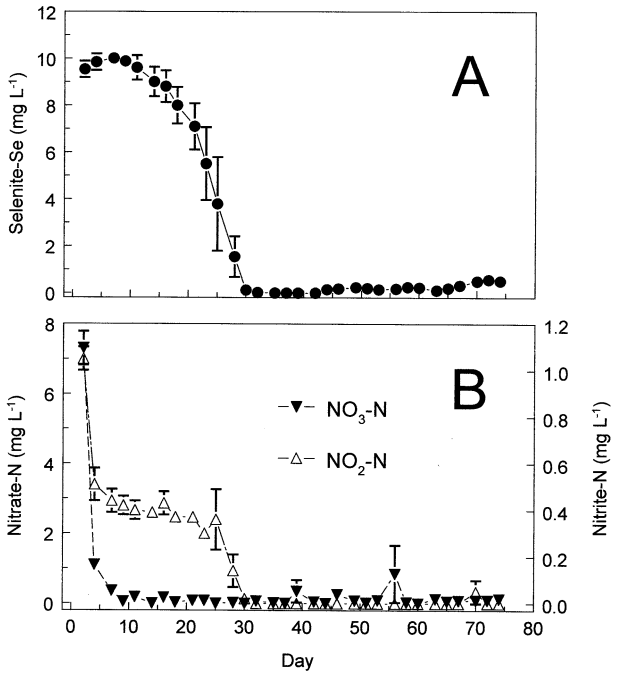

Fig. 1. Selenite (A), nitrate and nitrite (B) content of column effluents.

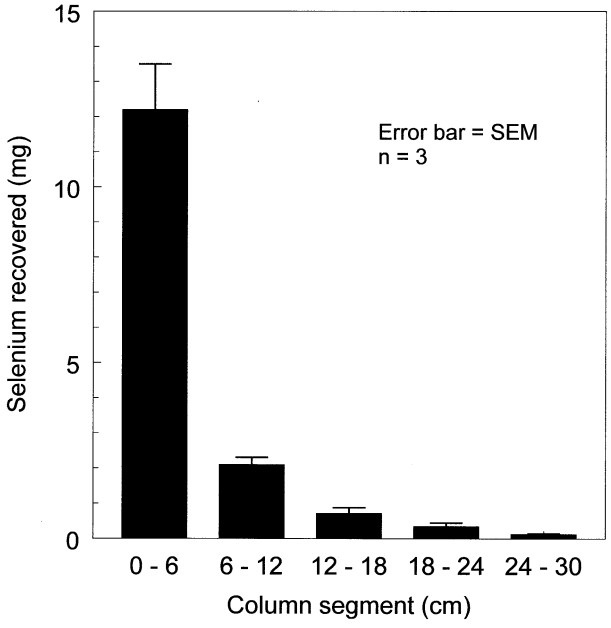

Fig. 2. Total selenium deposited in the sand columns. Distances are from the influent end of the column. $n = 3$. Error bar is SEM.

amount of nitrate-N in column effluents declined to below 1 mg L^{-1} N in the first week of operation and the levels remained low, averaging 0.10 ± 0.03, for the remainder of the study (Fig. 1B). This represents a removal efficiency of better than 99% during the last 65 days of the study. A transient buildup of nitrite in the column effluents that lasted for the first 28 days of the study was observed (Fig. 1B). During this 28-day period the amount of nitrite in the column effluents averaged 0.43 ± 0.06 mg L^{-1}. After the 28th day of the study, the amount of nitrite in column effluents declined to very low, usually undetectable, levels (Fig. 1B).

Selenite was also removed by the vegetable-oil-based biobarrier though there was a delay before the removal became effective. The amount of selenite-Se in the column effluents remained high, at more than 9 mg L^{-1}, for the first 14 days of the study. After this initial 2 week period, the amounts of selenite in the effluents began a steady decline that lasted until the 28th day of the study. From day 28 until the end of the study the amount of selenite-Se in the column effluents averaged 0.20 ± 0.04 mg L^{-1} (Fig. 1A). Thus, about 98% of the selenite present in the influent was removed by the biobarrier during the latter part, from day 28 onward, of the study. No selenate was detected in column effluents.

Selenium remaining in the columns. During the study a 5–6 cm area near the influent end of the sand columns developed a reddish color. Spectral analysis of the reddish material showed an absorbance peak of about 550 nm. This spectrum is similar to but slightly lower than those reported for purified nanospheres of red selenium isolated from *Sulfurospirillum barnesii* and *Bacillus selenitireduncens* by Oremland et al. [21]. Elemental selenium, Se^0, often forms a reddish-colored precipitate and the presence of a reddish color in the columns would suggest that particles of insoluble red amorphous Se^0 were accumulating [3].

Further evidence that immobilized selenium had accumulated in the columns was obtained when the columns were disassembled at the end of the study. During disassembly the sand in the columns was removed in five 6 cm segments and the total amount of selenium that had accumulated in each segment was determined by atomic absorption analysis. Results show (Fig. 2) that a large amount of selenium, 12.2 ± 1.3 mg, was present in the first segment of the columns, nearest to the inlet end.

Dimethylselenide (DMSe) and dimethyldiselenide (DMDSe) in column effluents. No DMDSe was detected during the study but DMSe began to accumulate in effluent line vapor traps during the fourth week of operation of these columns and the amount that accumulated continued to increase until week 9 when the amount produced peaked (Fig. 3). The total amount of DMSe lost from the columns as a vapor was approximately 39 ng over the course of the

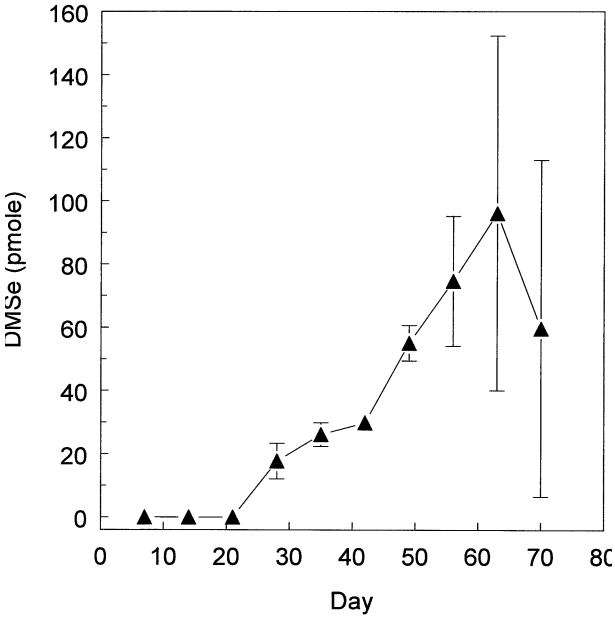

Fig. 3. Accumulation of DMSe in gas traps placed in the effluent lines of the columns. $n = 3$. Error bar is SEM.

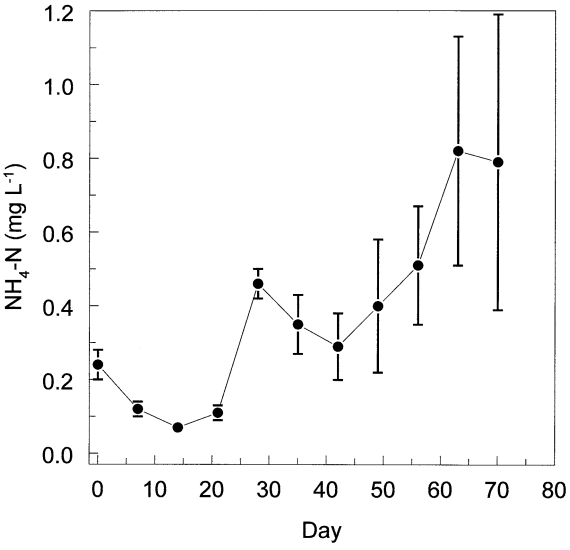

Fig. 4. Ammonia in column effluents. $n = 3$. Error bar is SEM.

study. No attempt was made to quantify the amount of DMDSe or DMSe that was present in the liquid effluent.

Ammonia, COD, and pH. Ammonia was detected in the column effluents and the amount of ammonia-N in the effluents increased by 5- to 6-fold during the course of the study (Fig. 4). At the start of the study, during the first 10 days, ammonia-N averaged about 0.14 mg L^{-1}, which increased to 0.82 mg L^{-1} by day 63. Over the course of the study ammonia-N averaged about 0.4 mg L^{-1}; this is less than the amount of ammonia-N (1 mg L^{-1}) that was included in the influent buffer.

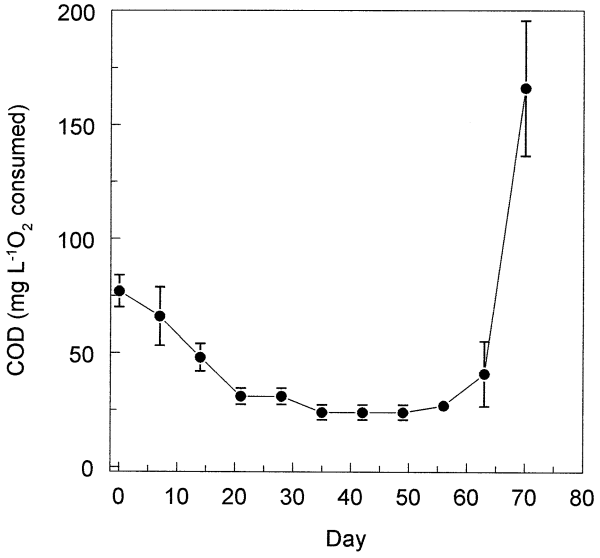

Fig. 5. COD of column effluents. $n = 3$. Error bar is SEM.

COD provides a measure of the amount of oxygen required to degrade compounds, mostly organic, present in an effluent water sample. Effluent waters from denitrifying biobarriers often have an elevated COD [13]. The COD of the column effluents averaged 51 ± 13 mg L^{-1} O_2 over the course of the study. Values ranged from a low of 24 to a peak of 166 mg L^{-1} O_2 (Fig. 5). CODs in this range would provide sufficient carbon to stimulate microbial activity [2]. Thus, under in situ conditions, the influence of the biobarrier would likely extend downstream of the actual barrier provided that other nutrients were not limiting.

The pH of the columns' effluent waters were monitored throughout the study. At the start of the study the pH of effluents was 6.9 ± 0. Effluent pH rose slowly during the study such that by the end the average pH was 7.3 ± 0. An increase in pH is typical of denitrifying biobarriers.

Nitrite formation in microcosms. It was interesting that the timing of the buildup of nitrite in the column effluents correlated so closely with the time that selenite was present in the column effluents. This suggests that the presence of selenite in the columns may have influenced the formation of nitrite. More evidence of a relationship was observed in microcosm studies, where nitrite also formed when selenite was present. The

anaerobic microcosms were inoculated with a ditch soil extract and contained 0, 10, or 100 mg L^{-1} selenite-Se. The microcosms were incubated for 7 days and after the incubation microcosms that contained no selenite had formed 5.3 ± 3.3 mg L^{-1} nitrite-N while microcosms that contained 10 and 100 mg L^{-1} selenite-Se formed 59.9 ± 14.8 and 75.8 ± 3.8 mg L^{-1} nitrite-N respectively. This suggests that selenite, at concentrations of 10 mg L^{-1} or greater, interferes with the reduction of nitrite and can cause nitrite to accumulate under denitrifying conditions. Lesser concentrations of selenite may also cause nitrite to accumulate but were not tested.

Discussion

These studies used a laboratory model of a biobarrier to demonstrate that an in situ biobarrier containing vegetable oil should be capable of removing both 20 mg L^{-1} nitrate-N and 10 mg L^{-1} selenite-Se from flowing groundwater. In these laboratory biobarriers the amount of nitrate-N in the water was reduced to 0.10 mg L^{-1}, 1% of the USEPA drinking water standard for nitrate of 10 mg L^{-1}. At the same time the amount of selenite in column effluents was reduced to 0.20 mg L^{-1}, an amount well below the USEPA drinking water standard of 5 mg L^{-1}. The accumulation of a reddish precipitate in the initial part of the column and the immobilization of selenium in the sand indicates the formation of insoluble Se^0 and indicates that a reduction process is involved in the removal of selenite. Microorganisms detoxify selenite by reduction to elemental selenium and deposit the Se^0 as reddish granules in the cytoplasm, the periplasmic space or outside of the cell [4, 26].

The formation of nitrite in biobarriers used for remediation would be undesirable. Based on the recommendations of the USEPA the maximum contaminant level (MCL) for nitrite in drinking water is 1.0 mg L^{-1}, whereas for nitrate the MCL is 10 mg L^{-1} [32]. While column samples from only one time period exceeded the nitrite MCL in this study, the amount of nitrate applied to the columns was not as large as might be encountered under some environmental conditions [28, 34]. If large amounts of nitrate are present in the waters being remediated for selenite then an unacceptable amount of nitrite might form for a period of time.

There are a number of possible explanations for the transient formation of nitrite. There may have been competition for electrons by the microbial processes involved in the reduction of nitrite and selenite and it might be this competition for electrons that slows the reduction of nitrite when selenite is present. Nitrite and selenite may also be reduced by the same enzyme in some bacteria [1] and selenite could slow nitrite reduction by competing for the active site of the enzyme. There are a number of other possible explanations that could cause nitrite to form in the presence of compounds such as selenite [12].

During the study 31.7 ± 3.3 mg of selenite-Se was applied to the columns in the influent water. About half of the applied selenium, 48.9% or 15.5 ± 1.8 mg, was recovered from the sand columns when they were disassembled (Fig. 2). An additional 10.5 ± 0.8 mg, or 33%, of the total applied to columns was recovered as selenite-Se collected in the effluents. Almost all, 7.7 ± 0.8 mg, of the selenite-Se recovered in the column effluents was recovered in the first 28 days of the study (Fig. 1A). This leaves about 5.7 ± 1.0 mg, or 18%, of the total applied to the column unaccounted for. The amount of selenium lost as vapor, approximately 39 ng, would represent an insignificant amount of the total selenite applied to the column. No attempt was made to quantify the amount of selenium as DMSe that was present in the effluent liquid phase. It is possible that the amount was significant and may be large enough to account for some or much of the 5.7 mg of selenium missing in the mass balance. Based on the Henry's law constant for DMSe the concentration of DMSe in the aqueous phase of the effluent could be up to 14 times greater than the concentration present in the vapor phase [6]. No selenate was detected in the column effluents.

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